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Biological control of *Fusarium oxysporum* f. sp. *melonis* causing muskmelon wilt

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ABSTRACT

Five different antagonists viz. *Aspergillus niger*, fluorescent *Pseudomonas*, non-pathogenic *Fusarium oxysporum*, *Trichoderma harzianum* and *T. viride* were evaluated as seed treatment against *Fusarium* wilt of muskmelon caused by *Fusarium oxysporum* f. sp. *melonis*. All the antagonists showed rhizosphere competence and reduced the number of colony forming units of *F. oxysporum* f. sp. *melonis*. Non-pathogenic *F. oxysporum* and *T. harzianum* were the most effective in reducing the wilt incidence by 77.2 and 74.5 per cent and pathogen population by 54.5 and 53.8 per cent, respectively at 30 days after sowing. Seed treatment with antagonists resulted in increased peroxidase levels in roots of plants.

Key words: Biological control,*Fusarium oxysporum* f. sp *melonis*, muskmelon wilt.

Fusarium wilt of muskmelon caused by *Fusarium oxysporum* f. sp. *melonis* was of minor importance in Punjab until late nineties when this wilt became destructive and the crop failed in most of the muskmelon growing region of the state. (Anonymous, 1998). Being a soil-borne disease it was difficult to be managed on account of the prevalence of the inoculum in the soil. The use of systemic fungicides and the potential of biological agents for the management of Fusarium wilt diseases have been reported (Larkin and Fravel, 1998). The present investigations were undertaken with an objective to find a suitable biocontrol agent, which could be a part of the intergrated management strategy for the disease.

MATERIAL AND METHODS

Collection and identification of antagonists

Surveys of different agro climatic zones of the state were conducted and samples of wilted muskmelon plants were collected. One hundred and thirty three isolates were obtained from roots of these plants, out of which *F. oxysporum* f. sp.*melonis* was found to be pathogenic whereas *Aspergillus niger*, fluorescent *Pseudomonas*, non-pathogenic *Fusarium oxysporum, Trichoderma harzianum* and *T. viride* were antagonistic to *F. oxysporum* f.sp. *melonis*.

Rhizosphere competence of antagonists and their effect on *F. oxysporum* f. sp. *melonis* population and muskmelon wilt

Seeds of muskmelon var. Punjab Sunehri were treated with talc based formulation of biocontrol agents so that the soil contained 1.8x 10^5 cfu/g of each fungal antagonist and 10^8 cell/g of bacterial (flourescent *Pseudomonas)* antagonist. The seeds (10 seeds/pot) were sown at a depth of 1.5 cm in pots filled to two-third capacity (1.5 kg) with field soil i.e. steam sterilized soil mixed with inoculum of *F. oxysporum* f. sp. *melonis* (1.8x10^5 cfu/g soil). Rhizosphere competence of biocontrol agents was assayed by recording their population density (cfu/g soil) at an interval of 15 days up to 45 days after sowing.

Population dynamics on *F. oxysporum* f. sp. *melonis* was recorded and population density of the pathogen was determined at an interval of 15 days up to 45 days after sowing. Reduction in population density was calculated 45 days after sowing. Reduction in population density was calculated 45 days after sowing.

Observations on seed germination were recorded after 7 days of sowing and per cent disease incidence and disease intensity were recorded after 30 days and percent disease reduction was calculated.

Effect of seed treatments with bio-agents on peroxidase activity in muskmelon roots

One month old muskmelon plants raised from seeds treated with antagonists were uprooted and their roots were washed with running tap water. Roots were then put in polythene bags, placed in refrigerator. Analysis for enzyme activity and protein content was performed on the same day.

Preparation of enzyme extract

Three roots per treatment were used. Each root was weighed and ground in a pre chilled pestle and mortar with glass capillaries and 3 ml ice cold 0.1 M Tris-HCl buffer of pH 7.5 containing $5x10^{-3}$ M-2merceptoethanol. The extract was centrifuged at 7800-8000 rpm for 10 min. the supernatent, thus obtained was used as enzyme extract for determination of peroxidase activity and protein content. All these operations were carried at 4°C.

Peroxidase assay

The enzyme was assayed by following the appearance of brown colouration resulting from guaiacol oxidation to tetra-guaiacol in the presence of hydrogen peroxide (Shannon et al, 1966). The reaction mixture contained 3ml of 0.05 M guaiacol in 0.1 M sodium phosphate buffer (pH6.5), 0.1 ml of enzyme extract (ten times diluted sample) and 0.1 ml of 0.8 M H_2O_2. The reaction mixture without H_2O_2 used as blank. The reaction was initiated by the addition of H_2O_2 and the rate of change of absorbance was recorded at 470nm after 30 sec up to 3 min. Enzyme activity was expressed as the increase in absorbance at 470nm $min^{-1}g^{-1}$ root tissue.

Estimation of total proteins (Lowry *et al.* 1951)

Five ml of a regent (freshly prepared by mixing 50 ml of 2% sodium carbonate in 0.1N NaOH and 1 ml of 1% copper sulphate in 2% sodium potassium tartarate) was added to 0.1 ml of enzyme extract. The contents were mixed well and allowed to stand for 10 min at room temperature. Folin phenol reagent (0.5 ml) diluted with distilled water (1:1) was added to it. Blue coloration, so obtained was read after 30 min at 520nm taking only water and reagents as blank. The concentration of total protein was calculated from the standard curve prepared by using bovine serum albumin (BSA) in the range of 50-250 µg. Enzyme activity was expressed as the increase in absorbance at 520nm min^{-1} mg^{-1} protein.

RESULTS AND DISCUSSION

Rhizosphere competence of antagonists

Rhizosphere competence of antagonists showed that the initial population density of fungal antagonists ($1.8x10^5$ cfu/g seed) was reduced considerably at 15 DAS but thereafter there was a gradual decline (Table 1). Non-pathogenic *F. oxysporum* population was reduced to 46.5 x 10^3 cfu/g soil at 45 DAS. Similarly, the population of antagonists showed a downward trend with the minimum population density of 30.7 x 10^3 cfu/g soil

Tabel 1. Rhizosphere competence of antagonists in soil at different days of sowing (DAS)

Treatment	Population density (cfu/g soil)			
	Initial	15 Das	30 Das	45 Das
Aspergillus niger	$1.8x10^5$	$53.6x10^3$	$45.4x10^3$	$30.7x10^3$
Fluorescent *Pseudomonas*	$1.0x10^8$	$7.4x10^7$	$34.7x10^6$	$19.5x10^6$
Non-pathogenic *F. oxysporum*	$1.8x10^5$	$62.5x10^3$	$57.3x10^3$	$46.5x10^3$
Trichoderma harzianum	$1.8x10^5$	$59.6x10^3$	$51.1x10^3$	$35.9x10^3$
Trichoderma viride	$1.8x10^5$	$54.8x10^3$	$45.4x10^3$	$32.7x10^3$

Tabel 2. Effect of seed treatment by various antagonists on *Fusarium oxysporum* population at different days after sowing (DAS)

Treatment	Population density of *F. axysporum* (cfu/g soil)				Reduction of cfu (%)
	Initial	15 DAS	30 DAS	45 DAS	at 45 DAS
Aspergillus niger	$1.5x10^4$	$6.8x10^2$	$5.9x10^2$	$4.2x10^2$	34.7
Fluorescent *Pseudomonas*	$1.5x10^4$	$6.5x10^2$	$5.8x10^6$	$3.7x10^2$	47.0
Non-pathogenic *F. oxysporum*	$1.5x10^4$	$6.4x10^2$	$4.2x10^3$	$2.9x10^2$	54.5
Trichoderma harzianum	$1.5x10^4$	$6.4x10^2$	$4.9x10^3$	$3.0x10^2$	53.8
Trichoderma viride	$1.5x10^4$	$6.6x10^2$	$5.9x10^3$	$4.0x10^2$	38.8
Untreated inoculated control	$1.5x10^4$	$6.9x10^2$	$6.1x10^3$	$6.5x10^2$	---

of *A. niger* recovered at 45 DAS. The bacterial antagonists also showed a decline in its population from $1x10^8$ cfu/g soil at 15 DAS and 19.5 x 10^6 cfu/g soil at 45 DAS.

Gullino *et al.* (1995) reported a sharp decline in the population of antagonistic *F. oxysporum* from initial amount ($5x10^5$ cfu/g soil) to a stable concentration of 10^3 to 10^4 cfu/g after six to ten weeks. Parke *et al.* (1991) observed that the population densities of low temperature growing isolates of *Pseudomonas* spp. were continuously reduced without regard to temperature, whereas, Loper *et al* (1984) had demonstarted that survival ability of *Pseudomonas* was stable at low temperatures only . Jayaraj and Ramabadran (1999) also reported a decline in the population density of *T. harzianum* in soil upto 90 DAS but the maintenance of soil mositure at 10 per cent was ideal for the efficient growth and multiplication of *Trichoderma* propagules.

Effect of antagonists on *F. oxysporum* f. sp. *melonis* population

The results (Table 2) revealed that the counts of colony forming units of *F. oxysporum* recovered from the soil decreased with time from the initial concentration of 1.5 x 10^4 cfu/g soil. At 15 DAS in the untreated control, a population density of 6.9 x 10^2 cfu/g soil was recorded whereas a minimum population density of 6.4 $x10^2$ cfu/g soil was observed where non-pathogenic *F. oxysporum* was used as biocontrol agent. Further decrease in the population was recorded upto 45 DAS in all the treatments. However, the population in control was more or less stable, falling in the range of 6.4 x 10^2 - 6.9 x 10^2 cfu/g soil. Minimum population density of 2.9 x 10^2 cfu/g soil of *F. oxysporum* f.sp. *melonis* was observed in the non-pathogenic *F. oxysporum* followed by *T. harzianum* ($3x10^2$ cfu/g soil), fluorescent *Pseudomonas* (3.7 x 10^2 cfu/g soil), *T.viride* ($4.0x10^2$ cfu/g soil) and *A. niger* (4.2 x 10^2 cfu/g soil).

Therefore, the reduction in the colony forming units of pathogenic *F. oxysporumn* at 45 DAS was maximum (54.5%) in seeds treated with non-pathogenic *F. oxysporum* followed by *T. harzianum.* It was least in seed treatment with *A.niger* (34.7%).

Takehara *et al* (2003) also reported suppression in the population of pathogenic *F. oxysporum* f. sp. *spinaciae* (1x105 cfu/g soil) by addition of non-pathogenic *F. oxysporum* in soil. Considerable reduction in *F. oxysporum* f. sp. *melonis* population in rhizosphere was reported by Chattopadhyay and Sen (1996). They observed 87.3 and 77.8 per cent reduction of colony forming units at 10 weeks after sowing in *A. niger* and *T. viride* treatments, respectively.

Effect of antagonist on muskmelon wilt

The data (Table 3) revealed that the germination of muskmelon was significantly improved in all the treatments except *A. niger* treated seeds on which germination percentage was statistically at par with control. The maximum germination of 89.3 per cent was recorded with fluoresecent *Pseudomonas* followed by non-pathogenic *F. oxysporum* (88.7%), *T. harzianum* (87.3%) and *T.viride* (84.7%). Bio-agent treatment significantly reduced the disease

Tabel 3. Effect of antagonists on germination of muskmelon, wilt incidence and peroxidase activity at 30 days after sowing (DAS)

Biocontrol agent (5g/Kg seed)	Germination (%)	Disease incidence(%)	Disease reduction (%)	Peroxidase activity	
				ΔE/min/g root ± SD	ΔE/min/g Protein ± SD
Aspergillus niger	76.7 (61.3)*	19.3 (26.0)	41.4	99.3±29.3	438.4±91.5
Fluorescent *Pseudomonas*	89 3 (71.5)	10 4 (18.8)	68 3	186.5±44.2	669.9±84.8
Non-pathogenic *F. oxysporum*	88 7 (70 4)	7.5 (15.9)	77.2	214.9±45.5	724 2±77.2
Trichoderma harzianum	87 3 (70.6)	8 4 (16.8)	74.5	198.6±31.9	719.1±61.1
Trichoderma viride	84.7 (67.3)	11.7 (20 0)	64.3	184.7±21.4	680.2±87.0
Control	60.7 (51.1)	32.9 (35 0)	---	48.3±10.8	189.3±68.0
CD (p=0.05)	(10.2)	(2 3)	(3.1)		

* Values in parentheses are transformed values

incidence. Minimum disease incidence(7.5%) was recorded with non-pathogenic *F. oxysporum* followed by *T. harzianum* (8.4%), fluorescent *Pseudomonas* (10.4%), *T. viride* (11.7%) and *A. niger* (19.3%). This resulted in a maximum reduction of 77.2 per cent in disease incidence with non-pathogenic *F. oxysporum* followed by *T. harzianum* (74.5%).

Aspergillus niger was ineffective than other biocontrol agents tested. Sen (2000) reported 81 per cent wilt control by treating muskmelon seeds with *Kalisena* SD, a bio formulation of *A. niger* (AN 27). Protection against Fusarium wilts by application of non pathogenic strains of *F. oxysporum* is a well studied phenomenon (Olivian and Alabouvette, 1999). Soil-borne non pathogenic *F. oxysporum* strain showed ability to protect melon plants against pathogenic isolates of *F. oxysporum* f. sp. *melonis*. Fluorescent *Pseudomonas* and non pathogenic *F. oxysporum* isolates suppressed Fusarium wilt of cucumber when added to soil together. *Pseudomonas* was also reported to reduce Fusarium wilt of cucumber pathogen after seed treatment (Wei *et al* 1992). *T. harzianum* also showed a considerable reduction in wilt incidence. Sivan and Chet (1989) reported *T. harzianum* strain T-35 to control Fusarium wilt of melons caused by *F. oxysporum* f.sp. *melonis*.

Effect of antagonist on peroxidase activity in muskmelon roots

The results (Table 3) revealed that the disease incidence and the peroxidase activity was greatly influenced by biocontrol agents. Maximum peroxidase activity of 214.9 ± 45.5 (ΔE/min/g root ± SD) was recorded in the treatment with non pathogenic *F. oxysporum* where the per cent disease incidence was low (7.5%). High peroxidase activity (198.6±31.9) and low disease incidence (8.4%) were also recorded in *T. harzianum*. All the antagonists significantly reduced the disease and enhanced the peroxidase activity in comparison to control.

The peroxidase activity calculated on the basis of per mg protein in the roots also indicated a similar trend with maximum peroxidase activity of 724.2 ± 77.3 (ΔE/min/g root±SD) in seed treatment with non pathogenic *F. oxysporum*. Muskmelon seedlings raised from bio agent treated seeds showed much higher peroxidase activity over check. Angappan *et al* (1996) reported that muskmelon seedlings raised from *A. niger* formulation treated seeds showed 56 per cent reduction of *F. oxysporum* f. sp. *melonis* and 58 per cent higher peroxidase activity.

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